Membrane transport proteins: not just for transport anymore

Jonathan D. Kaunitz

Medical Service, West Los Angeles Veterans Affairs Medical Center, Department of Medicine, UCLA School of Medicine, and CURE: Digestive Diseases Research Center, Los Angeles, California

Digitalis glucosides have been used for their cardiotonic properties for several centuries. It was not until the 1950s that the molecular target of these compounds was identified as the Na-K-ATPase. Until recently, the only action attributed to digitalis at its molecular target was the inhibition of the Na-K-ATPase catalytic cycle, with consequent inhibition of ion transport and phosphorylation.

In the 1950s, evidence accumulated that endogenous digitalis-like compounds were present in animals (2). The putative "natriuretic hormone" that is secreted with dietary salt loading, and is implicated in the pathogenesis of essential hypertension, was attributed to a family of endogenous digitalis glycoside compounds termed endogenous ouabain (EO), which are elevated in subjects with essential hypertension. Although the pathogenesis of EO-associated hypertension was assumed to relate to inhibition of Na-K-ATPase-mediated ion transport, comparison of the relative potencies of EO with regard to hypertension production and ATPase inhibition paradoxically revealed an inverse, rather than the predicted positive correlation (9). Furthermore, although all digitalis-like compounds inhibit Na-K-ATPase-mediated transport, they differ considerably in terms of their hypertensive effects. These findings do not support the prevailing hypothesis that the pathophysiological effect of digitalis glycosides results from inhibition of the Na-K-ATPase transport activity. Nevertheless, recent studies firmly implicate the Na-K-ATPase ouabain binding site as the molecular target of the hypertensive effects of these compounds (1). To integrate these findings, investigators have hypothesized that the Na-K-ATPase can act as a signal transducer, in addition to its role as an ion pump, regulated by nanomolar concentrations of digitalis-like compounds (6, 13, 14).

The concept that transport proteins may subserve functions other than facilitating the movements of solutes across membranes is not novel. Indeed, the Na-K-ATPase has been implicated in gene regulation in the control of cell growth and proliferation in cardiac myocytes (8). The cystic fibrosis transmembrane regulator has numerous regulatory functions in addition to anion transport (7, 12). Nevertheless, relatively few studies have addressed the notion that one inhibitor, acting at a different location in the same protein, termed "long-range linkages." Inesi et al. (5) observed that the specific Ca\textsuperscript{2+} ATPase inhibitor thapsigargin inhibited two different enzyme functions, at different molecular locations, independently of catalysis. It is likely that ouabain interacts in a similar fashion with the Na-K-ATPase, inhibiting alternative functions independently of enzyme cycling. Thus a biophysical basis exists for specific inhibition of the P-type ATPases producing effects independent of catalysis and transport.

In this issue of the American Journal of Physiology-Renal Physiology, Oweis et al. (12a) examined the effect of ouabain on the renal-derived cell line, LLC-PK1, demonstrating that a concentration of ouabain too low to inhibit Na-K-ATPase-mediated transport or to raise intracellular [Na\textsuperscript{+}] will nevertheless affect the function and expression of the epithelial Na\textsuperscript{+}/H\textsuperscript{+} exchanger NHE-3. The authors test the hypothesis that the renal Na-K-ATPase is a target for EO at a concentration that does not affect its transport function. The authors demonstrate that 100 nM ouabain inhibited recovery of intracellular pH and H\textsuperscript{+} gradient-driven \textsuperscript{22}Na\textsuperscript{+} uptake in LLC-PK1 cells after a 12- to 24-h ouabain exposure, which had no effect on [Na\textsuperscript{+}]. In subsequent studies, the authors demonstrated that the same ouabain exposure decreased the abundance of NHE-3 RNA and protein. The remainder of the paper was devoted to the study of the mechanism of this downregulation, with a demonstration that ouabain decreased binding of the transcription factors TR and Sp1 to the NHE3 promoter, by studying the involvement of caveolin-1 with P-11 and C2-9 cells, and using inhibitors to show that the tyrosine kinase c-Src and PI3 kinase, enzymes shown to be activated by the ouabain-Na-K-ATPase interaction (13, 14), are involved in the downregulation.

The importance of the study is the demonstration that ouabain concentrations that do not affect Na-K-ATPase transport function can nevertheless affect expression of key transport proteins implicated in renal salt handling and indirectly in hypertension pathogenesis, such as NHE-3 through genomic signaling mechanisms. These effects of ouabain are only beginning to be studied in epithelial cells and thus have major implications into the understanding of the regulation of epithelial transport. One potential drawback of the paper is that the investigators performed several experiments with cardiac myocytes, the preferred model system for most of their prior studies (8), using relatively high ouabain concentrations. This nonepithelial system is probably not the best model for the study of NHE3 function or expression. Nevertheless, LLC-PK1 cells are widely accepted models of proximal tubular cells and indeed were one of the first systems in which NHE3 was pharmacologically identified before its cloning (3).

A more fundamental concern is that although nanomolar concentrations of ouabain may indeed activate a signaling cascade eventuating in gene regulation (8), the circulating concentrations of EO are in the picomolar range (4, 10). Furthermore, the notorious resistance of the rat α1 subunit to ouabain, combined with its abundant expression in the nephron (11), also casts doubt regarding its functional role of EO in hypertension pathogenesis (4). These concerns have been addressed by testing the effect of mutations of the Na-K-ATPase ouabain binding site on the development of hypertension in mice, which have convincingly shown that ouabain binding site affinity strongly correlates with hypertension (1). Further-

Address for reprint requests and other correspondence: J. D. Kaunitz, Bldg. 114, Suite 217, West Los Angeles VAMC, Los Angeles, CA 90073 (e-mail: jake@ucla.edu).

http://www.ajprenal.org

more, drugs that selectively antagonize EO have been suggested for the treatment of essential hypertension (1a).

This paper represents the convergence of studies conducted in the fields of membrane biology, transport physiology, plant and medicinal chemistry, molecular cell biology, biophysics, and clinical medicine. The recognition that small concentrations of endogenous substances similar or identical to plant-derived bioactive compounds can produce unexpected yet important effects on membrane proteins will undoubtedly be explored further, yielding fresh insight into fundamental biological processes in general and epithelial transport in particular.

REFERENCES


